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SOLID SUPPORTED REACTIONS IN ENVIRONMENTAL ANALYSES:

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INTRODUCTION:

Solid phase processes are an approach to simplifying and automating analytical procedures for the determination of organic compounds from aqueous matrix. The most common technique is that of adsorption/desorption on reverse phase column chromatography which serves to isolate and concentrate organics from aqueous matrix. Isolation and concentration, however, are frequently insufficient to achieve the sensitivities for environmental analysis and derivatization is frequently required. As a rule such analytical reactions are carried out in solution and off-line which complicates sample handling and makes automation more complex. Solid supported reaction were investigated to deal with problems of solution derivatization and of-line reactions (1-5). This work was based on the hypothesis that analytical methods using complete solid phase sample preparation technology will lead to a more facile development of automated procedures.

Determination of the chlorophenoxy acetic acid herbicides and their phenolic breakdown products usually involves derivatization of the acidic functionality. Methylation or pentafluorobenzylation are the usual reactions used to prepare the derivatives . We investigated pentafluorobenzylation of these analytes on a solid support of a XAD-2 a macroreticular styrene/divinylbenzene cross linked copylmeric resin. In conjunction with this derivatization procedure we also developed in-line chromatographic separation of derivatized analytes from each other and from derivatives of interferences.

EXPERIMENTAL:

Apparatus: The pentafluorobenzyl (PFB) derivatives of pure analytes were determined on a Helwett-Packard (H-P) 5790 GC equipped with a pulse linearized ECD and a J & W fused capillary column DB-1, 30 M X 0.321 MM with film thickness 0.25 µM. The output of the detector was monitored on a H-P 3390A recording integrator. Hydrogen was used as a carrier gas with linear velocity of 62 cm/sec at 180°C and 10 % methane in argon was used as a make-up gas at a flow rate 15 ml/min.

Reagents: Pentafluorobenzyl bromide (PFBBr) was purchased from Caledon Laboratories, Georgetown, Ontario. The macroreticular resin, XAD-2, a cross-linked copolymer of styrene/divinylbenzene was obtained from BDH Laboratories, Toronto, Ontario and was cleaned and stored as previously

described (2,3). Florisil and basic alumina were purchased from Supelco (Canada) Oakville Ont. Disposable 1 ml Supelclean columns used for packing the Florisil and basic Alumina semi-preparative column and a vacuum module were also purchased from Supelco (Canada) Oakville Ont. Solvents were purchased from the usual commercial suppliers, such as Fisher, BDH and Aldrich Canada. Pure analytes were obtained from the E.P.A Repository.

All the glass-ware was silylated by standard procedures. Glass-ware and plastic-ware was washed with methylene dichloride, methanol, acetonitrile and dried prior to use.

Preparation of PFB Derivatives: Pentafluorobenzyl derivatives of the pure analytes were prepared by stirring of the organic acid in acetone with PFBBr with K_2CO_3 as the base. Reaction work-up consisted of evaporating the acetone, extracting the residue with $CH2Cl_2$ and finally washing the organic phase with distilled water. The PFB products were purified by thin-layer chromatography (3).

Derivatization and Isolation: Two hundred mg of XAD-2 was added to a 16X100 mm screw cap vial and wetted with 100 uL of acetonitrile. Four mL 0.1 M Phosphate buffer at pH 7.4 containing analyte was added to these vials followed by 100 uL of PFBBr in hexane (1/9 v/v). The reaction mixture was shaken for 2 hours in a water bath at 40°C. The resin was isolated by filtration in a 5 mL Supelclean cartridge and washed with distilled water. After the interstitial water, was removed by suction 100 µL of acidified 2,2 dimethoxypropane was added and allowed to remain in contact with the resin for 15 minutes. Excess scavenging reagent and products of hydrolysis (acetone and methanol) were removed with a gentle stream of nitrogen at room temperature. The cartridge was then inserted linked in series to a 1 mL cartridge containing Florisil and a 1 mL cartridge containing basic alumina. Derivatized phenols were eluted from the link with hexane/toluene (99/2 v/v). The Florisil and Alumina columns were split and PFB-2,4 dichlorophenoxy acetate was eluted with hexane/acetone (99/1 v/v)

Gas Chromatography: Samples were analyzed by gas chromatography with electron capture using the column described above and the following following temperature conditions: 180-215°C at 4°C/min; 215°C to 300°C at 20 °C with a 2 minute hold at 300°C.

Results and Discussion:

In the herbicide problem there are several distinct issues that need to be dealt with in the development analytical techniques based on pentafluorobenzylation using solid supported reactions. Firstly there are two distinct groups of analyte that are involved: the carboxylic acids and the corresponding phenols that are the environmental breakdown products. Derivatization

must therefore, **in principle**, be carried out under alkaline conditions to ensure ionization of both the weak (phenol) and strong (carboxylic) acid. Secondly, there are numerous interferences that exist even in the laboratory matrix. These are fatty acids which are simultaneously derivatized under alkaline conditions to electrophoric derivatives and endogenously electrophoric material in found in plastic-ware.

The simultaneous derivatization of chlorophenoxy acetic acids and the corresponding phenols was investigated as an approach to the development of a simultaneous determination of all pertinent analytes. The model compounds were 2,4 dichlorophenoxy acetic acid, 2,4-dichlorophenol, 2,4,5-trichlorophenol and pentachlorophenol. The reactivity and the chromatographic properties of these analytes were found in many instances to be atypical.

Table I. Derivatives Recovered from different reaction and chromatographic conditions

	Aqueous Phase		Normal Phase used for chromatography	
Analytes	0.1 N NaOH	0.1M Phosphate Buffer pH 7.4	Florisil	Alumina
Phenols[1]	PFB-ether	no product	PFB-ether	PFB-ether
2,4 DiClPh	PFB-ether	no product	PFB-ether	PFB-ether
2,4,5 TriClPh	PFB-ether	no product	PFB-ether	PFB-ether
PentaClPh	PFB-ether	no product	PFB-ether	PFB-ether
Acids[2]	PFB-ester	PFB-ester	PFB-ester	PFB-ester
2,4 DiClPA	no product	PFB-ester	PFB-ester	no product

1. eg Cannabinoids (1,2,5); indoleamines (1)
2. Straight chain Carboxylic acids 9to 24 carbon atoms (4); carboxylic acid metabolites of cannabinoids (2)

Derivatization of organic acids in heterogeneous aqueous/organic systems requires pH that is sufficient to ionize the acid. Accordingly at alkaline conditions both the phenols and 2,4 dichlorophenoxy acetic acid should be expected to react. In

our hands, the pentafluorobenzyl (PFB) ester of the acid was not recovered at alkaline conditions whereas PFB ethers of 2,4-dichlorophenol, 2,4,5-trichlorophenol and pentachlorophenol were formed and isolated (Table I). The reason for this is not clear but it was possible that under alkaline conditions here may have been hydrolysis of 2,4 dichlorophenoxy acetic acid to 2,4-dichlorophenol. However, a PFB ether of the corresponding phenol was not recovered from XAD-2 supported pentafluorobenzylation of 2,4 dichlorophenoxy acetic acid at alkaline pH. The problem was not investigated further at that point, as we found that simultaneous derivatization of the analytes and recovery of PFB derivatives of all the analytes was possible if derivatization was carried out at pH 7.4. These were the only phenols that, in our experience, were derivatized at pH 7.4 and probably reflected the lowered pKa of this class of phenol.

Derivatization of analytes was necessary but insufficient for developing methods of high sensitivity. The organic acid matrix of lipophilic compounds that can interfere, even when limited to ordinary laboratory reagents and water, is quite complex. Separation of derivatized analyte from interferences was an essential consideration.

A primary requirement of any separation technique is effective transfer of analyte to the chromatographic phase. In the case of solid supported reaction such transfer involved two steps: elution from the resin in high yield followed by transfer to the normal phase without undue spreading of the derivative over the length of the column. This combination of requirements dictated the elution from the resin in the most lipophilic solvent possible and this required development of effective drying conditions.

After derivatization the reaction products remain adsorbed on a surface that is in turn coated with water. This makes elution with lipophilic solvents inefficient (Fig. 1 a) by inhibiting contact of eluant with the surface of the resin. As a result 50-70% is eluted in hexane and the remainder requires elution with more polar solvents. If the latter group of solvents were used to elute from the resin onto the normal phase then, not surprisingly, there was very no effective separation of derivatized analyte from interferences.

The first step of the separation problem was thus reduced to the drying of the resin. This is a standard requirement following adsorption of analyte from water using a reverse phase columns composed of alkylsilica or XAD-2. This is usually affected by heating or with vacuum in conjunction with a stream of nitrogen. This approach, however, cannot be used when dealing with the low molecular weight, and hence volatile, analytes and derivatives under investigation. Accordingly we tested a volatile water scavenger 2,2-dimethoxypropane to dry the resin

(Fig. 2). This proved to be effective after treatment of the resin and after evaporation at low temperature and at atmospheric pressure of 2,2-dimethoxypropane and it's hydrolysis products it was possible to elute all the analytes from the resin in hexane.

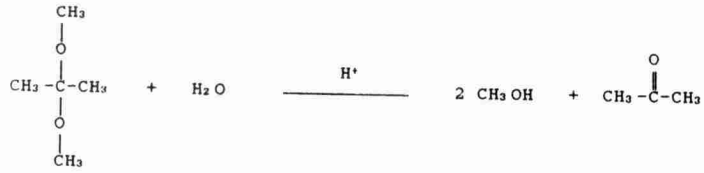

Figure 2. Reaction of 2,2 dimethoxypropane with water.

Florisil was not effective at separating the PFB derivatives of the phenolic analytes from the interferences since both these products eluted with hexane (Fig. 3). As a result a linked system using basic alumina as the normal phase was investigated. This phase allowed isolation of the PFB ethers in a reasonably clean isolate (Fig 4) 5 ng/mL. A difficulty was encountered with PFB-2,4 dichlorophenoxy acetate which could not be recovered from this normal phase (Table I). Thus the unexpected behaviour of 2,4 dichlorophenoxy acetic acid and it's PFB derivative necessitated rther modification of the linked system.

It was proposed that clean-up of the PFB ethers and PFB ester could be affected by a linked system which allowed lipophilic eluate from the Florisil to be transferred directly to a column of basic Alumina thus trapping the interferences and allowing the PFB ethers to elute. The link between the two normal phase columns was then broken and the PFB-2,4 dichlorophenoxy acetate was eluted from the Florisil with acetone in hexane (Fig 5).

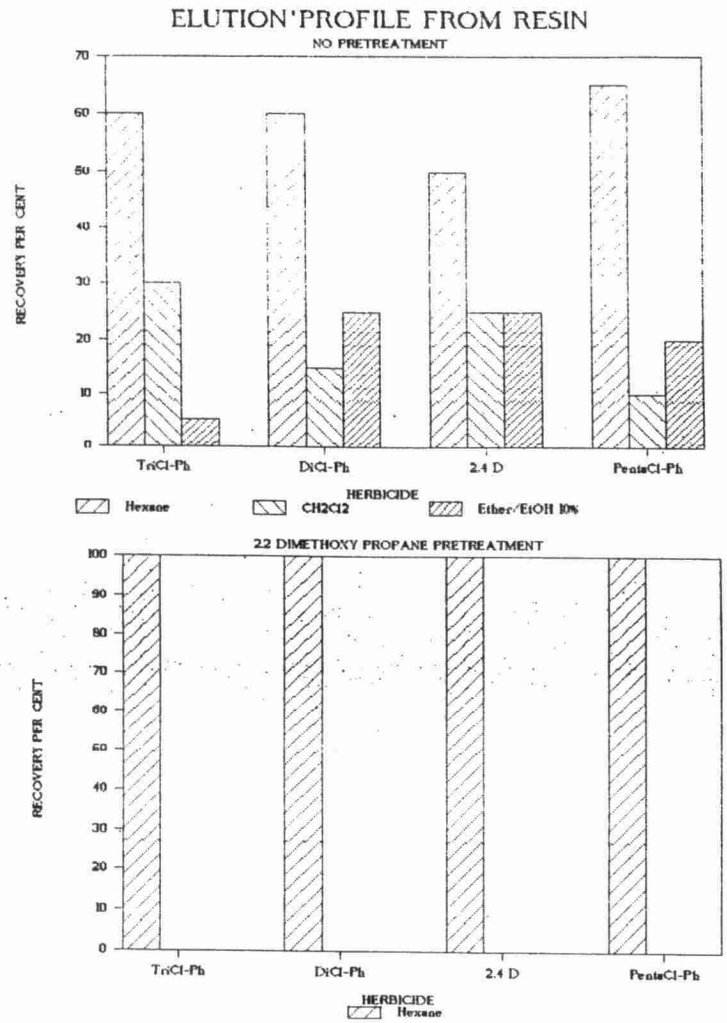

Figure 1. Elution profile of derivatized analytes from resin with A) no treatment; B) drying with 2,2-dimethoxypropane.

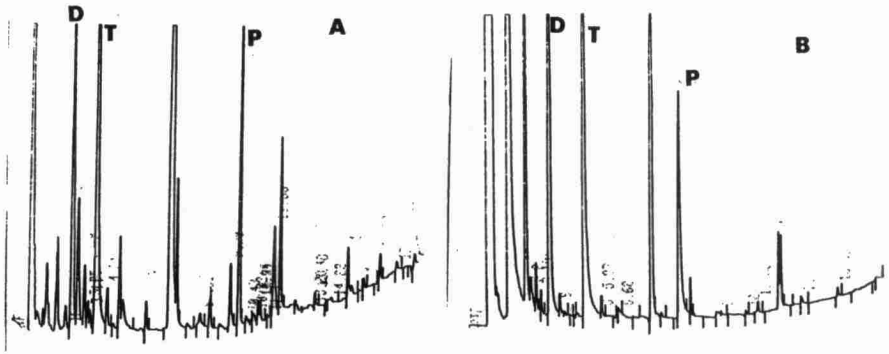

Figure 3. Clean-up of reaction for derivatization of phenols on XAD-2: A) Hexane eluate from Florisil; B) Hexane/ Toluene (99/1 v/v) eluate from Florisil/Alumina link. (For analysis of B tempreature program was started at 170°C to compensate for interferences in the solvent front). D = 2,4-dichlorophenol; T = 2,4,5-tricholorophenol and P = pentachlorophenol

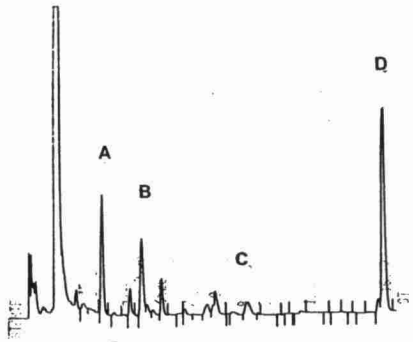

Figure 4. Determination of 5 ng/mL of polychlorinated phenols from water: PFB derivatives eluted in the Hexane/Toluene (99/1 v/v) eluate from Alumina.

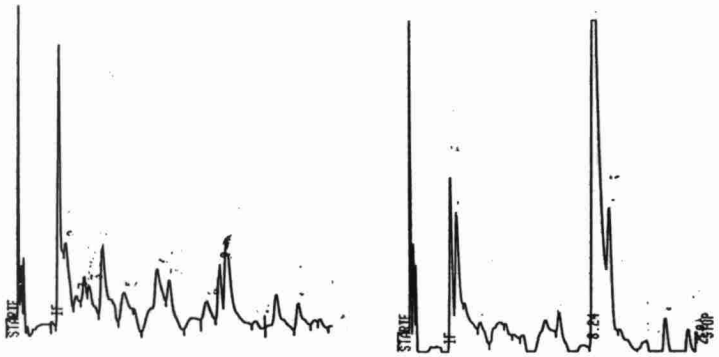

Figure 5. A) Hexane/Acetone (95/5 v/v) eluate of blank B) Hexane/Acetone (95/5 v/v) eluate of sample containing 2,4 Dichlorophenoxy acetic acid (50 ng/mL).

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